Highly Pathogenic Avian Influenza Surveillance in the Central Flyway

~2008 Summary Report~

for

The Central Flyway Council Technical Committee

Photo Courtesy of Bob Malbon

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USDA, APHIS, Wildlife Services

Wildlife Disease Surveillance and Emergency Response Program

Wildlife Services

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Introduction

The third year of surveillance for highly pathogenic avian influenza (HPAI) in wild, migratory birds concluded on March 31st. With the continued goal of early detection of HPAI in wild, migratory birds, the sampling effort was successful in establishing that the U.S. is free of HPAI, generating new information about avian influenza viruses circulating in waterfowl in the U.S., and in fostering cooperative relationships between wildlife agencies.

Surveillance for HPAI will continue in biological year 2009 (BY09), with some significant changes. Although sampling methods will remain unchanged, a significant decrease in funding for BY09 has caused a reduction in sampling effort. In particular, environmental sampling in all states and apparently healthy wild bird surveillance in level 3 states will be discontinued for BY09. All states (including level 3 states) are encouraged to continue to collect samples from morbidity and mortality events.

In the past couple of years, isolated incidents of mortalities attributed to HPAI H5N1 have been identified in various species of birds. Research also has indicated that prior exposure to North American low pathogenic H5 viruses provide some cross protection to HPAI (Pasick et al. 2007), which supports testing apparently healthy waterfowl as well as investigating morbidity and mortality events. Wild ducks, mallards in particular, have been implicated as long distance carriers of HPAI H5N1 (Keawcharoen et al. 2008). Recent genetic analyses have shown higher rates of intercontinental virus exchange than previously recorded (Koehler et al. 2008), further supporting surveillance in mallards, northern pintails, and other dabbling ducks.

Surveillance for HPAI H5N1 in wild birds continues to be important, because advances in wild, migratory bird health are being made and the epidemiology of the virus and its effect on wild bird populations, agriculture and human health remains unknown. National surveillance is providing information to expand our knowledge regarding the ecology of avian influenza and other diseases in wild birds and helping identify patterns of disease and predict areas of high risk. Surveillance is also helping to protect natural resources and American agriculture.

2008 Number of Samples and Collection Locations

The U.S. Interagency Strategic Plan calls for robust sampling of waterfowl and other species of wild birds in North America that represent the highest risk of being exposed to or infected with HPAI virus because of their migratory patterns or potential contact with species from areas in Asia with reported outbreaks. An emphasis was placed on active surveillance of ducks, geese, and shorebirds and passive surveillance of other birds through morbidity/mortality events. Sample sizes for each state were originally determined based on several criteria such as amount of wetland habitat, overwintering waterfowl population size, linear distance of shoreline, and waterfowl band recovery data. Once sample numbers were determined for each state, local expertise was used to determine sampling locations and priority species within each state. The majority of the samples (70%) were collected during the migratory season when birds are most likely to introduce the virus to the U.S. and when low pathogenic avian influenza circulates in waterfowl at higher prevalence. The remaining 30% of the samples was collected during the resident season to ensure that local

birds had not become infected via a pet bird introduction, illegal shipment of poultry or poultry products, or by humans.

Wild bird target sample size during the 2008 sampling season was increased slightly from the previous year (57,250 to 63,200) to better align sample size targets in each state and to increase statistical power for detecting HPAI. The increased overall sample size still emphasized quality of samples in areas of high risk and collection from specific species. Dabbling ducks and other groups of species considered competent carriers of the virus were again targeted for sampling.

Surveillance for HPAI was conducted in all four North American Flyways with corresponding activities in Canada and Mexico to ensure the goal of early detection in wild, migratory birds. Biological year 2008 began on April 1, 2008 and continued through March 31, 2009. During the year, 64,741 wild bird samples and 25,976 fecal samples were collected nationwide. This total includes the combined efforts of state wildlife agencies and various tribes that participated in surveillance under the direction and funding provided by USDA's Animal and Plant Health Inspection Service, Wildlife Services (WS). These numbers do not include samples collected by Department of Interior (DOI), states under contract with DOI, research activities, or other projects.

Cooperative sampling involving Wildlife Services, California Department of Fish & Game, U.S. Fish & Wildlife Service

Of the total number of wild bird samples, 20,567 were collected in the Atlantic Flyway, 17,484 in the Mississippi Flyway, 13,711 in the Central Flyway, 12,541 in the Pacific Flyway, and 438 in Hawaii and the Pacific Islands. The Central Flyway accounted for approximately 21% of the overall wild bird sampling effort. Wild migratory bird collection locations are depicted in Figure 1.

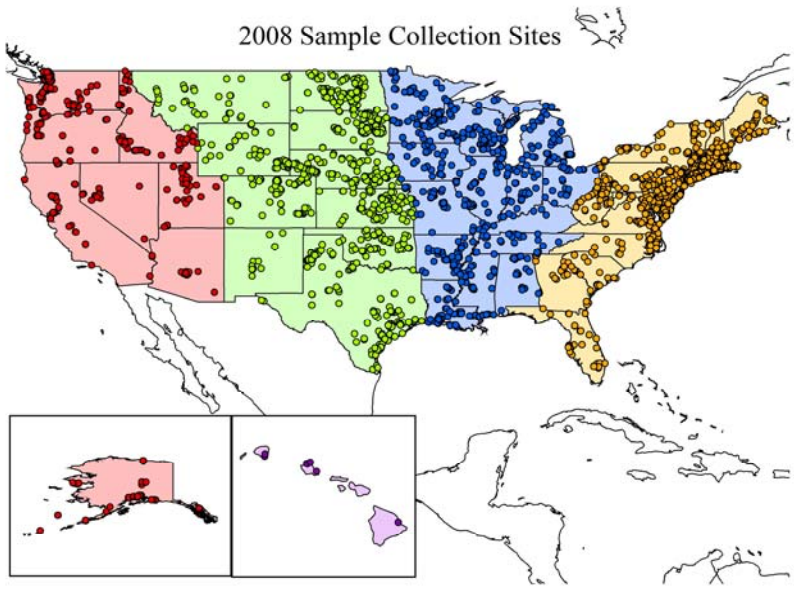

Figure 1. Collection Sites of Wild Bird Samples Collected in 2008.

Sample locations in the Central Flyway were distributed geographically as depicted in Figure 2. Surveillance in the Central Flyway states provided coverage to intercept migratory birds moving southward from Alaska and Canada as well as coverage of migratory birds potentially moving HPAI viruses north from South or Central America. Even though HPAI has not been detected in the Western Hemisphere, sample locations in 2008 provided excellent geographic distribution throughout the entire Central Flyway.

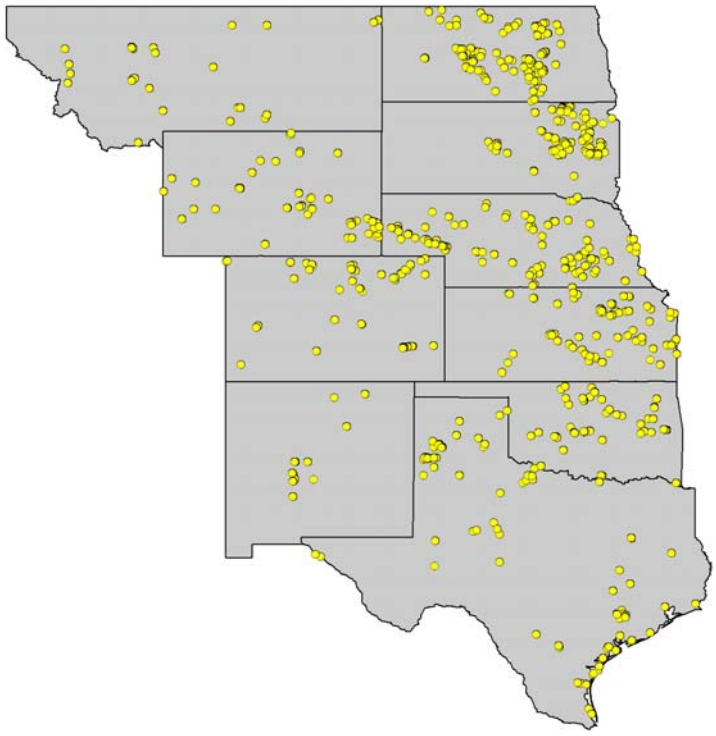

Figure 2. Collection Sites of Wild Bird Samples in the Central Flyway in 2008.

In addition to collecting samples directly from wild, migratory birds, environmental sampling for detecting HPAI was also implemented in the Central Flyway. Environmental sampling consisted of the collection of fresh fecal material deposited by migratory waterfowl and shorebirds. Specific collection sites included locations representative of habitat used by large concentrations of priority species. Number of sites per state varied depending on the amount of suitable habitat and migratory bird presence and were collected in 8 Central Flyway states in 2008 (Figure 3). The Central Flyway effort accounted for 24% (6,145 actual samples) of the national fecal sampling effort.

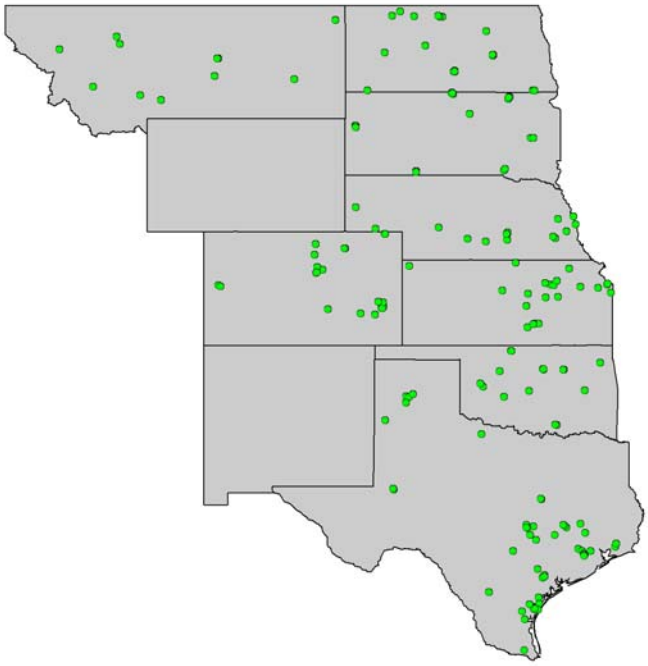

Figure 3. Collection Sites for Environmental Sampling in the Central Flyway in 2008.

Diagnostic Testing of Wild Bird Samples

All wild bird samples were sent for testing to one of 45 diagnostic laboratories that are part of the National Animal Health Laboratory Network (NAHLN). The NAHLN is part of a national strategy to coordinate animal health diagnostic services in the US by positioning the National Veterinary Services Laboratories (NVSL) as the lead US animal health laboratory and allowing select laboratories operated by State, Federal and university officials to cooperate in foreign animal disease surveillance and related services. NVSL serves as the national reference laboratory by providing other diagnostic laboratories with animal disease information and technical guidance as well as confirmatory testing of foreign animal diseases.

Wild bird samples were tested individually, and each sample was initially screened for type A influenza with matrix H5 and H7 real-time reverse transcriptase polymerase chain reaction (rRT-PCR) assays. If the matrix assay was positive for type A influenza, subtyping was conducted with the H5 and H7

specific rRT-PCR assays. Diagnostic testing at the NAHLN laboratories was completed within 48 hours of receipt of samples at the laboratory.

A new H7 assay was used for detecting H7 viruses in 2008. The primers and probes for the new assay allowed detection of the H7 lineage of virus that is currently circulating in wild birds as well as H7 viruses that have been isolated historically from the live bird market. The new assay has a broader specificity than the one that had been used in the previous two years. As a result of the new assay, there were increased detections of H7 viruses.

NVSL conducted rRT-PCR on each sample that was forwarded to them as a presumptive H5 or H7 finding. Virus isolation was also conducted on each sample sent to NVSL by inoculating a suspension of each specimen into the embryos of chicken eggs to replicate the virus in order to determine the H and N subtypes of the virus. Genetic sequencing of H5 and N1 positive isolates was one method used to determine the pathogenicity of the virus. Additionally, 4 to 8-week old disease-free chicks were inoculated with all H5 isolates to determine pathogenicity. All H5N1 detections in 2008 were considered low pathogenic avian influenza (LPAI) because the avian influenza (AI) virus was never lethal for 6 or more of the 8 chickens that were inoculated, and all H5 isolates were genetically related to North American strains of known LPAI viruses.

All environmental samples were submitted to WS' National Wildlife Research Center where diagnostic testing was conducted based on the approved NAHLN protocol. Fecal samples were pooled in the laboratory with up to 5 samples and screened for the presence of AI viruses using rRT-PCR matrix assays. Positive pools were then screened for H5 and H7 by rRT-PCR and H5 or H7 positive pools were sent to NVSL for confirmation.

Functional Groups and Collection Strategies

Flexibility was built into the national surveillance plan to encourage local expertise to guide sampling within each state while standardizing procedures and methods used across the U.S. Collectors chose from 1 of 5 wild bird collection strategies depending on the most appropriate strategy for the area and the species targeted. Environmental sampling was only conducted by WS employees. An emphasis was placed on investigating morbidity/mortality events, but live capture, hunter harvest and environmental sampling comprised the largest portion of the collection strategies. This balanced approach proved important in providing credible data to show an absence of HPAI in the U.S.

During the 2006 biological year, an emphasis was placed on sampling individual species of birds. This allowed us to learn more about avian influenza ecology in wild, migratory birds without compromising the goal of early detection of HPAI. This data was used to guide surveillance efforts in 2007 and 2008, and led to a functional group approach. Grouping wild birds by related species or guilds relies on the assumption that birds with similar behavior and feeding habits have comparable probability of contracting and spreading HPAI and other avian influenza viruses. It also facilitated examination of results and trends at a larger scale rather than examining individual species.

Percentages from 2008 sampling of functional groups for the Central Flyway are depicted in Figure 4. Dabbling ducks (Genus *Anas* and *Aix sponsa*) accounted for 76% of the overall effort, followed by geese & swans (Subfamily Anserinae) at 17% and diving ducks (Subfamily Aythyinae) at 6%. Justification for the large percentage of dabbling ducks being sampled is due to the relative

facility in collecting samples from hunter check stations in comparison to shorebirds and other species where live trapping was the only active surveillance option available. Historical banding operations of dabbling ducks and population size also factored in to the large percentage represented in Figure 4. Functional groups of shorebirds (Order Charadriiformes), gulls, terns, and alcids (Families Laridae and Alcidae) and other (all birds not represented in another functional group) accounted for 1%, <1%, and <1% of the sample total, respectively.

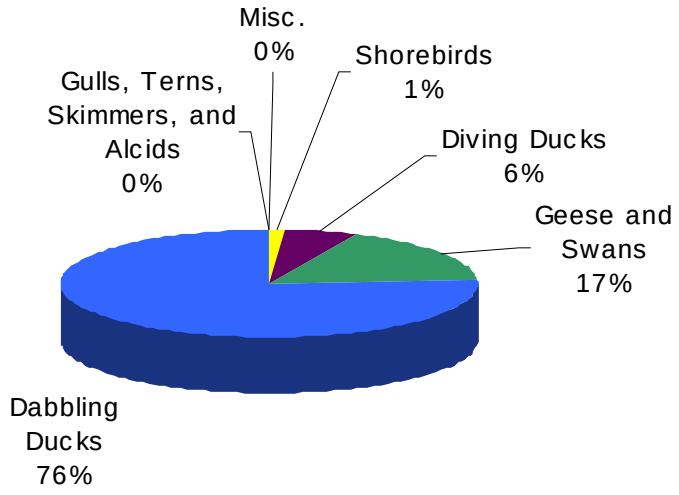

Figure 4. Percent Samples Collected by Functional Group for HPAI surveillance in the Central Flyway in 2008.

The U.S. Interagency Strategic Plan identifies 5 collection strategies that can be utilized in collecting samples for HPAI detection (morbidity/mortality, live wild bird, hunter harvest, sentinel species, and environmental). Decisions on which collection strategies to use were made through flyway plans and at the state level, except for environmental sampling.

Although morbidity/mortality event sampling made up <1% of the total percentage of samples collected in the Central Flyway (Figure 5), the strategy remained a very important method for sampling for HPAI in wild birds. Recent literature has shown that many species of wild birds will likely succumb to a natural HPAI infection, and natural immunity and pre-exposure to LPAI viruses might afford some level of protection. Wild birds that do not act as natural reservoirs of LPAI viruses may be more likely to die from infection of the virus, thus making collection of samples from morbidity/mortality events important.

As seen in Figure 5, hunter harvest and live wild bird sampling collectively accounted for 69% of the total number of samples. Environmental sampling (31%) also played an important role in the overall surveillance in the Central Flyway.

Within the Central Flyway, 10,247 hunter harvested wild birds and 3,437 live wild birds were captured for HPAI surveillance. Of the 1,418 live wild birds captured by WS' employees, 13 birds (0.9%) died as a result of capture. While any loss during live wild bird collection is to be avoided, the mortality rate due to live capture was extremely low.

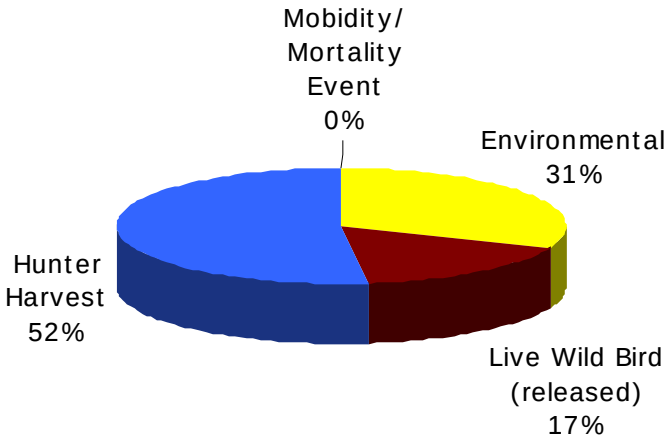

Figure 5. Percent of Samples by Collection Strategy in the Central Flyway in 2008.

Results

Surveillance resulted in no detections of HPAI in either wild bird or environmental samples. Of the 64,741 wild bird samples submitted to NAHLN laboratories for screening, 945 individual samples from 270 locations in 46 states were sent to NVSL for confirmation. Of the 945 samples, NVSL confirmed that 575 samples collected from 213 locations in 42 states were LPAI H5 positive. NVSL also confirmed that 117 samples were H7 positive.

Within the Central Flyway, 10 NAHLN labs were responsible for testing the wild bird samples. Through the screening process, 118 samples from 10 states collected at 64 locations screened H5 positive or suspect (based on cycle-threshold values) at a NAHLN laboratory. These samples were sent to NVSL for confirmation. One hundred eleven samples from 61 locations were confirmed LPAI H5 positive by NVSL (Figure 6). Thirty-two samples from 7 states collected at 17 locations screened H7 positive or suspect at a NAHLN laboratory. Twenty nine samples from 15 locations in 7 states in the Central Flyway were confirmed by NVSL as H7 positive (Figure 6). Once again, HPAI was not detected in any of the wild bird samples submitted for HPAI testing.

The following species from the Central Flyway were confirmed H5 positive at NVSL: American Green-winged Teal, American Wigeon, Blue-winged Teal, Canada Goose, Common Goldeneye, Gadwall, Lesser Snow Goose, Mallard, Northern Pintail, Northern Shoveler, and Ross's Goose. The following species were confirmed H7 positive: American Green-winged Teal, Blue-winged Teal, Mallard, Northern Pintail, and Ring-necked Duck.

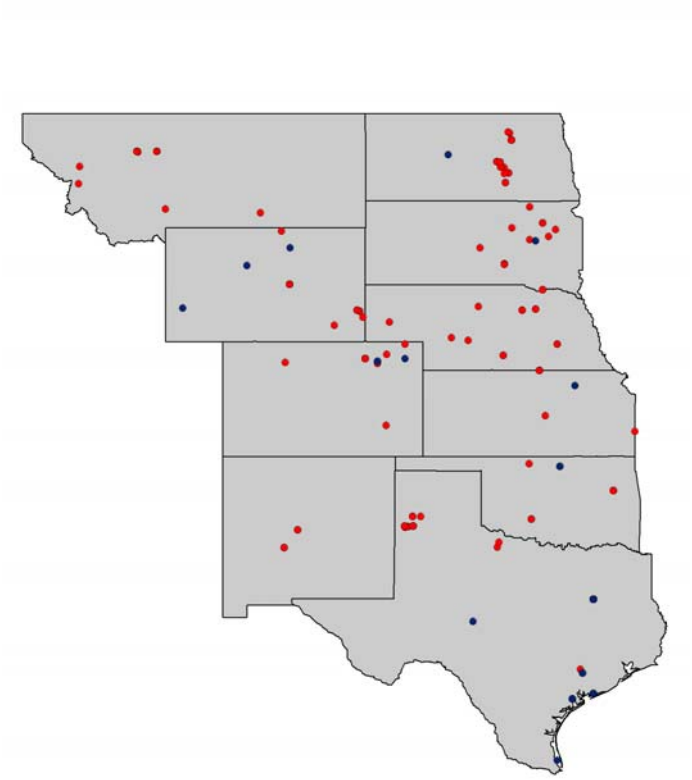

Figure 6. NVSL confirmed LPAI H5 (Red) and H7 (Blue) Positive Sites in the Central Flyway in 2008.

Of the 25,976 fecal samples collected during 2008, there were 246 matrix positive pools. Of the matrix positive pools, 4 pools from 2 states (Iowa and Oregon) screened H5 positive and were sent to NVSL for confirmation. One of the 4 pools was confirmed positive. The positive pool came from the Pacific Flyway, and there was no confirmed H5 or H7 positive pools in the Central Flyway.

Changes in HPAI surveillance for 2009

Due to decreased funding for the 2009 biological year, some significant changes were made. Total number of samples to be collected during the 2009 sampling season will be decreased from 85,000 to 44,000 samples to emphasize sampling in areas of higher risk and collection from specific species. Environmental (fecal) sampling will not be conducted or be included as part of the early detection of HPAI in wild, migratory birds in BY09. This reduction alone accounted for 25,000 fewer samples. Level 3 states (2 states out of 10 in the Central Flyway) will not be funded to collect samples from apparently healthy wild birds. Wild bird surveillance will continue in the 36 remaining level 1 and level 2 states, including 8 Central Flyway states. Sample sizes in level 1 & 2 states will also be slightly decreased from 2008 due to budget shortages. WS employees and state wildlife agencies in level 3 states are encouraged to investigate morbidity/mortality events and collect samples when deemed appropriate. WS will continue to cover diagnostic expenses for these samples if sent to approved NAHLN labs.

Literature Cited

Keawcharoen, J., D. van Riel, G. van Amerongen, T. Bestebroer, W. E. Beyer, R. van Lavieren, A. D.M.E. Osterhaus, R. A. M. Fouchier, and T. Kuiken. 2008. Wild Ducks as Long-Distance Vectors of Highly Pathogenic Avian Infl uenza Virus (H5N1). Emerging Infectious Diseases 14(4):600-606.

Koehler, A. V., J. M. Pearce, P. L. Flint, J. C. Franson, and H. S. Ip. 2008. Genetic evidence of intercontinental movement of avian influenza in a migratory bird: the northern pintail (*Anas acuta*). Molecular Ecology 17:4754-4762.

Pasick, J., Y. Berhane, C. Embury-Hyatt, J. Copps, H. Kehler, K. Handel, S. Babiuk, K. Hooper-McGrevy, Y. Li, Q. M. Le, S. L. Phuong. 2007. Susceptibility of Canada Geese (*Branta Canadensis*) to Highly Pathogenic Avian Influenza Virus (H5N1). Emerging Infectious Diseases 13(12):1821-1827.